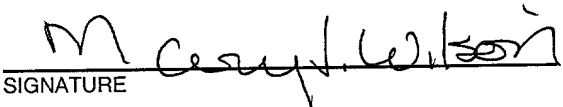


FORM PTO-1390 (REV 11-98)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 620-123
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) 09/701747 (To Be Assigned)
INTERNATIONAL APPLICATION NO. PCT/GB99/01743	INTERNATIONAL FILING DATE 3 June 1999	PRIORITY DATE CLAIMED 3 June 1998
TITLE OF INVENTION ION CHANNELS		
APPLICANT(S) FOR DO/EO/US WOOD et al.		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
<p>1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.</p> <p>2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.</p> <p>3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</p> <p>4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</p> <p>5. A copy of the International Application as filed (35 U.S.C. 371(c)(2)).</p> <p>a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</p> <p>b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau.</p> <p>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</p> <p>6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</p> <p>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under <u>PCT Article 34</u>.</p> <p>a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</p> <p>b. <input checked="" type="checkbox"/> have been transmitted by the International Bureau.</p> <p>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</p> <p>d. <input type="checkbox"/> have not been made and will not be made.</p> <p>8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (U.S.C. 371(c)(3)).</p> <p>9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</p> <p>10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</p> <p>Items 11. To 16. Below concern document(s) or information included:</p> <p>11. <input type="checkbox"/> An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.</p> <p>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.</p> <p>13. <input checked="" type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</p> <p>14. <input type="checkbox"/> A substitute specification.</p> <p>15. <input type="checkbox"/> A change of power of attorney and/or address letter.</p> <p>16. <input checked="" type="checkbox"/> Other items or information. PTO-1449/ International Search Report <input type="checkbox"/> This application is entitled to "Small entity" status. <input type="checkbox"/> "Small entity" statement attached.</p>		

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.51) (To Be Assigned) 09/701747		INTERNATIONAL APPLICATION NO. PCT/GB99/01743		ATTORNEY'S DOCKET NUMBER 620-123	
17. <input checked="" type="checkbox"/> The following fees are submitted:				CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 C.F.R. 1.492(a)(1)-(5)): -- Neither international preliminary examination fee (37 C.F.R. 1.482) nor international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO\$1000.00 -- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO\$860.00 -- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO\$710.00 -- International preliminary examination fee paid to USPTO (37 C.F.R. 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)\$690.00 -- International preliminary examination fee paid to USPTO (37 C.F.R. 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)\$100.00					
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$	860.00
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 C.F.R. 1.492(e)).				\$	130.00
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	53	-20 =	33	X	\$18.00
Independent Claims	5	-3 =	2	X	\$80.00
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)					\$270.00
TOTAL OF ABOVE CALCULATIONS =				\$	2014.00
Reduction by 1/2 for filing by small entity, if applicable. Small entity status must also be asserted. (Note 37 C.F.R. 1.9, 1.27, 1.28).					0.00
SUBTOTAL =				\$	2014.00
Processing fee of \$130.00, for furnishing the English Translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 C.F.R. 1.492(f)).					0.00
TOTAL NATIONAL FEE =				\$	2014.00
Fee for recording the enclosed assignment (37 C.F.R. 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. 3.28, 3.31). \$40.00 per property				+	\$ 0.00
Fee for Petition to Revive Unintentionally Abandoned Application (\$1240.00 - Small Entity = \$620.00)					\$ 0.00
TOTAL FEES ENCLOSED =				\$	2014.00
				Amount to be:	
				refunded	\$
				Charged	\$
a. <input checked="" type="checkbox"/> A check in the amount of \$2014.00 to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. 14-1140 in the amount of \$_____ to cover the above fees. A duplicate copy of this form is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-1140. A duplicate copy of this form is enclosed. d. <input type="checkbox"/> The entire content of the foreign application(s), referred to in this application is/are hereby incorporated by reference in this application.					
NOTE: Where an appropriate time limit under 37 C.F.R. 1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO: NIXON & VANDERHYE P.C. 1100 North Glebe Road, 8 th Floor Arlington, Virginia 22201 Telephone: (703) 816-4000					
				 SIGNATURE	
				Mary J. Wilson NAME	
				32,955 REGISTRATION NUMBER	
				December 4, 2000 Date	

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION OF

WOOD et al.

Atty. Ref.: 620-123

Serial No.: 09/701,747

Group Art Unit:

Filed: January 29, 2001

Examiner:

For: ION CHANNELS

* * * * *

June 27, 2001

RESPONSE TO NOTIFICATION TO COMPLY

Hon. Commissioner of Patents
and Trademarks
Washington, DC 20231

Sir:

This is in response to the Notice to Comply dated
April 27, 2001, in the above. The following comments are
offered.

REMARKS

Favorable consideration of this application is
respectfully requested.

The computer readable copy of the Sequence Listing
submitted herewith is the same as the paper copy of the
Sequence Listing as originally filed.

An early and favorable Action on the merits is
requested.

Respectfully submitted,

NIXON & VANDERHYE, P.C.

By Mary J. Wilson
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09/701747

528 Rec'd PCT/PTO 04 DEC 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

WOOD et al.

Atty. Ref.: 620-123

Serial No. (To Be Assigned)

Group:

National Phase of PCT/GB99/01743

Filed: December 4, 2000

Examiner:

For: ION CHANNELS

* * * * *

December 4, 2000

Assistant Commissioner for Patents

Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

Prior to calculation of the filing fee and in order to place the above identified application in better condition for examination, please amend the claims as follows:

IN THE CLAIMS

Claim 5, line 2, change "any one of the preceding claims" to --claim 1 or 2--.

Claim 10, line 2, change "any one of claims 5 to 9" to --claim 5--.

Claim 21, line 2, change "any one of claims 5 to 10" to --claim 5--.

Claim 24, line 2, change "any one of claims 5 to 10" to --claim 5--.

Claim 25, lines 1-2, change "any one of claims 21 to 23" to --claim 21--.

Claim 26, line 2, delete "or claim 25".

Claim 27, lines 1-2, change "any one of claims 1 to 4" to --claim 1 or 2--.

Claim 28, line 4, change "any one of claims 5 to 10" to --claim 5--.

Claim 36, line 4, change "any one of claims 1 to 4" to --claim 1 or 2--.

Claim 37, line 3, change "any one of claims 1 to 4" to --claim 1 or 2--.

WOOD et al.
Serial No. (To Be Assigned)

REMARKS

The above amendments are made to place the claims in a more traditional
format.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: Mary J. Wilson
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ION CHANNELSTechnical field

5 The present invention relates to proton or acid-sensing ion channels and nucleic acid encoding the same. It further relates to methods and materials for generating and using these.

10 Prior art

Acidosis accompanies many painful inflammatory and ischaemic conditions. The pain caused by acids is thought to be mediated in part by H⁺-gated cation channels present in sensory neurons. Thus H⁺-gated cation channels are of interest, *inter alia*, in the treatment of inflammation or other painful conditions e.g. through the use of targeted analgesics.

20 One H⁺-gated channel (ASIC, for acid-sensing ionic channel) has been cloned from rat (Waldmann et al. (1997) Nature 386, 6621, p173-177. This belongs to the amiloride-sensitive Na⁺ channel/degenerin family of ion channels (see Waldmann and Lazdunski 1998, Current
25 Biology 8: 418-424).

Heterologous expression of ASIC induces an amiloride-sensitive cation (Na⁺ > Ca²⁺ > K⁺) channel which is transiently activated by rapid extracellular
30 acidification. The biophysical and pharmacological properties of the ASIC channel closely match one of the H⁺-gated cation channels described in sensory neurons. However ASIC is also distributed widely throughout the brain.

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ART 34 AMDT

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Owing to the interest in H⁺-gated cation channels, it will be appreciated that the provision of a new channel, particularly one having one or more novel activities compared to those already available to the public, would provide a contribution to the art.

Disclosure of the invention

The present inventors have now cloned nucleic acid encoding a novel H⁺-gated cation channel from a rat sensory neuron cDNA library, and isolated and characterised the encoded protein. The nucleic acid sequence is shown as Seq ID No 1, while the encoded protein is Seq ID No 2. The channel, which is present in both spinal cord and throughout the central nervous system in subsets of neurons, is hereinafter referred to as "SPASIC".

Briefly the inventors used molecular cloning techniques to characterise the SPASIC protein. *In situ* DNA hybridisation and RNA Northern blotting showed that the new channel transcripts are present in dorsal root ganglion neurons and in central nervous system tissues. When expressed in transfected COS cells, the channel mediated both a transient and a sustained flow of current when exposed to extracellular acidity, e.g. of below pH 7.0.

SPASIC shares 43% sequence identity with ASIC (the amino acid sequence of which is shown as Seq ID No 3) and has similarities with other characterised ASIC-type proteins (see Figure 1). However SPASIC has unique properties. In particular it exhibits both a rapid, and then a sustained cation current in response to low pH. No H⁺-gated channel having these properties has been characterised

before.

Because of the expression of SPASIC in sensory neurons and the importance of proton-gated channels in pain and inflammation, this new channel is an analgesic drug target and has utility, *inter alia*, in screening techniques for identifying novel analgesic and anti-inflammatory agents.

10 Interestingly, its presence in subsets of central nervous system neurons also suggests an important role as a receptor or an autoreceptor involved in the regulation of neurotransmitter release or neuronal excitability or excitotoxicity. For instance, it is known that low pH is also found in synaptic vesicles, and the release of neurotransmitters is associated with the release of protons. Proton gated cation channels have been reported in isolated cells of the hypothalamus. Low pH has been found to be neuroprotective in some seizure models

20 (Valiseck et al. Brain Research 1995 Feb 13; 671(2):245-253) and to regulate synaptic efficacy (Velisek et al. Hippocampus 1998; 8(1):24-32). Thus the channel also has utility in screening for agents that regulate neurotransmitter release or neuronal excitability,

25 including the regulation of excitotoxicity and synaptic efficacy (e.g. in the context of learning and memory, cognition, perception, pain etc, depending *inter alia* on the location in which the channel may be expressed).

30 These and other aspects of the present invention will now be discussed in more detail.

Thus in a first aspect of the present invention there is provided an acid sensitive cation channel protein which

35 is capable of reversibly mediating (i) a rapid, and (ii)

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a sustained cation current.

Proteins, or polypeptides, of the present invention may be provided in recombinantly produced, isolated, enriched or cell-free form. They may be present in cells heterologously, which is to say that they do not naturally occur there, but have been introduced through human intervention.

By "acid sensitive" is meant that the cation permeability of the channel can be significantly increased by lowering the pH (increasing acidity, or reducing alkalinity).

The mediated current will occur in two phases, a "rapid" phase, and a "sustained" phase. Generally the rapid phase will last less than 500 milliseconds, more commonly less than about 200 milliseconds. The sustained phase will generally continue for the period in which the pH stimulus is maintained.

The two phase activity can be assessed by those skilled in the art without undue burden, for instance using voltage clamping techniques analogous to those described herein to analyse membranes or membrane preparations (e.g. from eucaryotic cells) into which the protein has been expressed or otherwise introduced. Some generally applicable methods are discussed in "Ionic Channels of Excitable Membranes" Hille, B. - Pub 1992, Sinauer.

Preferably the protein will have the electrophysiological and pharmacological characteristics of the SPASIC protein described herein. Preferably the protein comprises amino acid sequence Seq ID No 2.

In a further aspect of the invention there is disclosed a

SPASIC variant protein having ion channel activity and comprising an amino acid sequence having at least about 50%, or 60%, or 70%, or 80% homology, most preferably at least about 90%, 95%, 96%, 97%, 98% or 99% sequence identity with Seq ID No 2.

Ion channel activity may be tested as described above, using appropriate stimuli if required.

Similarity (or identity, or homology) may be as defined and determined by the TBLASTN program, of Altschul et al. (1990) *J. Mol. Biol.* 215: 403-10, which is in standard use in the art, or, and this may be preferred, the standard program BestFit, which is part of the Wisconsin Package, Version 8, September 1994, (Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA, Wisconsin 53711). BestFit makes an optimal alignment of the best segment of similarity between two sequences. Optimal alignments are found by inserting gaps to maximize the number of matches using the local homology algorithm of Smith and Waterman.

Homology may be over the full-length of the relevant sequence shown herein, or may be over a part of it, preferably over a contiguous sequence of about or greater than about 20, 25, 30, 33, 40, 50, 67, 133, 167, 200, 233 or more amino acids or compared with Seq ID No 2.

Thus a variant polypeptide in accordance with the present invention may include within the sequence shown in Seq ID No 2, a single amino acid or 2, 3, 4, 5, 6, 7, 8, or 9 changes, about 10, 15, 20, 30, 40 or 50 changes, or greater than about 50, 60, 70, 80 or 90 changes. In addition to one or more changes within the amino acid sequence shown, a variant polypeptide may include

additional amino acids at the C-terminus and/or N-terminus. Alternatively it may represent an active (as an ion channel) fragment of the protein e.g. a pore forming fragment.

5

Proteins or polypeptides of the present invention may be prepared by the expression of nucleic acids encoding therefor in appropriate host cells, as described in more detail hereinafter..

10

Thus in a further aspect of the present invention there is provided nucleic encoding a protein of the invention as described above.

15

Nucleic acid according to the present invention may include cDNA, RNA, genomic DNA and modified nucleic acids or nucleic acid analogs (e.g. peptide nucleic acid). Where a DNA sequence is specified, e.g. with reference to a figure, unless context requires otherwise the RNA equivalent, with U substituted for T where it occurs, is encompassed.

20

Nucleic acid molecules according to the present invention may be provided isolated and/or purified from their natural environment, in substantially pure or homogeneous form, or free or substantially free of other nucleic acids of the species of origin. Where used herein, the term "isolated" encompasses all of these possibilities.

25

The nucleic acid molecules may be wholly or partially synthetic. In particular they may be recombinant in that nucleic acid sequences which are not found together in nature (do not run contiguously) have been ligated or otherwise combined artificially. Alternatively they may have been synthesised directly e.g. using an automated

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synthesiser.

Thus in one embodiment of this aspect of the invention there is provided a nucleic acid encoding SPASIC e.g. a
5 nucleic acid encoding Seq ID No 2 (which nucleic acid sequence is set out as bases 292-1909 in Seq ID No 1). In another embodiment there is disclosed a sequence degeneratively equivalent to that sequence.

10 In another embodiment there is disclosed a nucleic acid which encodes a SPASIC variant as described above. Generally this will share homology or identity with the SPASIC encoding sequence in similar terms as are described in relation to the variant proteins above, but
15 wherein amino changes correspond to changes in codons or individual nucleotides.

Variants may include distinctive parts or fragment (however produced) corresponding to a portion of the
20 sequence provided. The fragments may encode particular functional parts of the polypeptide.

Equally the fragments may have utility in probing for, or amplifying, the sequence provided or closely related
25 ones. Suitable lengths of fragment, and conditions, for such processes are discussed in more detail below.

Also included are nucleic acids which have been extended at the 3' or 5' terminus with respect any of these
30 embodiments.

Preferred variant nucleic acids are those which, in addition to encoding SPASIC variants, are capable of hybridizing with at least one poly- or oligonucleotide of
35 sequence selected from sequence of SEQ ID No 1 under low

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stringency conditions, more preferably being capable of hybridizing with one or more of such sequences under high stringency conditions.

5 The expressions 'low stringency conditions' and 'high stringency conditions' will be understood by those skilled in the art, but are conveniently exemplified as set out in US 5202257, Col 9-Col 10. For instance, screening may initially be carried out under conditions, 10 which comprise a temperature of about 37°C or less, a formamide concentration of less than about 50%, and a moderate to low salt (e.g. Standard Saline Citrate ('SSC') = 0.15 M sodium chloride; 0.15 M sodium citrate; pH 7) concentration.

15 Alternatively, a temperature of about 50°C or less and a high salt (e.g. 'SSPE' = 0.180 mM sodium chloride; 9 mM disodium hydrogen phosphate; 9 mM sodium dihydrogen phosphate; 1 mM sodium EDTA; pH 7.4). Preferably the 20 screening is carried out at about 37°C, a formamide concentration of about 20%, and a salt concentration of about 5 X SSC, or a temperature of about 50°C and a salt concentration of about 2 X SSPE. These conditions will allow the identification of sequences which have a 25 substantial degree of homology (similarity, identity) with the probe sequence, without requiring the perfect homology for the identification of a stable hybrid.

Suitable conditions include, e.g. for detection of 30 sequences that are about 80-90% identical, hybridization overnight at 42°C in 0.25M Na₂HPO₄, pH 7.2, 6.5% SDS, 10% dextran sulfate and a final wash at 55°C in 0.1X SSC, 0.1% SDS. For detection of sequences that are greater than about 90% identical, suitable conditions include 35 hybridization overnight at 65°C in 0.25M Na₂HPO₄, pH 7.2,

6.5% SDS, 10% dextran sulfate and a final wash at 60°C in 0.1X SSC, 0.1% SDS.

Sequence variants which occur naturally may include alleles (which will include polymorphisms or mutations at one or more bases) or pseudoalleles. Also included within the scope of the present invention would be isogenes, or other homologous genes sharing the requisite identity with SPASIC. The term 'variant' nucleic acid as used herein encompasses all of these possibilities.

In a further aspect of the present invention there is provided a method of identifying and/or cloning a nucleic acid according to the present invention which method employs Seq ID No 1 or a distinctive fragment thereof.

In one embodiment, nucleotide sequence information provided herein may be used in a data-base (e.g. of expressed sequence tags, or sequence tagged sites) search to find homologous sequences, such as those which may become available in due course, and expression products of which can be tested for activity as described below.

In a further embodiment, a variant in accordance with the present invention is also obtainable by means of a method which includes:

- (a) providing a preparation of nucleic acid, e.g. from spinal cord,
- (b) providing a nucleic acid molecule having a nucleotide sequence shown in or complementary to Seq ID No 1 or a distinctive fragment thereof,
- (c) contacting nucleic acid in said preparation with said nucleic acid molecule under conditions for hybridisation of said nucleic acid molecule to any said gene or homologue in said preparation, and identifying said gene

or homologue if present by its hybridisation with said nucleic acid molecule.

Probing may optionally be done by means of so-called
5 'nucleic acid chips' (see Marshall & Hodgson (1998)
Nature Biotechnology 16: 27-31, for a review).

Nucleic acids of the invention may be amplified from
template DNA from cells using a specific DNA
10 amplification reaction with specific primers targeted to
amplify the DNA required, e.g. of SEQ ID No 1, e.g. from
genomic DNA, spinal cord or DRG cDNA, or mRNA templates,
e.g. by using polymerase chain reaction or, from RNA, by
using reverse transcription (RT) followed by polymerase
15 chain reaction (PCR) (see "PCR protocols; A Guide to
Methods and Applications", Eds. Innis et al, Academic
Press, New York, 1990).

Thus a method involving use of PCR in obtaining nucleic
20 acid according to the present invention may include:
(a) providing a preparation of nucleic acid, e.g. from
the CNS, or other appropriate tissue or organ,
(b) providing a pair of nucleic acid molecule primers
useful in (i.e. suitable for) PCR, at least one of said
25 primers having a sequence shown in or complementary to a
sequence shown in Seq ID No 1 or a distinctive fragment
thereof,
(c) contacting nucleic acid in said preparation with said
primers under conditions for performance of PCR,
30 (d) performing PCR and determining the presence or
absence of an amplified PCR product. The presence of an
amplified PCR product may indicate identification of a
variant.

35 Where it is desired to screen tissue derived libraries of

genomic DNA or cDNA or mRNA for DNA or RNA of the invention it will be convenient to use low then high stringency hybridization techniques as indicated above. Such screening may be carried out using hybridization probes targeted at all or part of SEQ ID No 1. Alternatively specific amplification primers (ie. PCR primers) as described above may be used to amplify homologous sequences native to the source DNA to be used as PCR template and the product DNA may be used as a specific probe when labelled to identify DNA of the invention from the genomic, cDNA or mRNA library material.

In each case, if need be, clones or fragments identified in the search can be extended. For instance if it is suspected that they are incomplete, the original DNA source (e.g. a clone library, mRNA preparation etc.) can be revisited to isolate missing portions e.g. using sequences, probes or primers based on that portion which has already been obtained to identify other clones containing overlapping sequence.

Distinctive fragments or oligonucleotides for use in probing or PCR may be selected prepared by those skilled in the art without burden in the light of the present disclosure. Typically they may be about 10 to 30 or fewer nucleotides in length (e.g. 18, 21 or 24). Generally specific primers are upwards of 14 nucleotides in length. For optimum specificity and cost effectiveness, primers of 16-24 nucleotides in length may be preferred. If required, probing can be done with entire restriction fragments of the gene disclosed herein which may be 100's or even 1000's of nucleotides in length.

For the purpose of identifying DNA coding for other ASIC-related proteins, it may be preferred to use probes and primers capable of hybridizing with those regions of the SEQ ID No 1 which are highly conserved between SPASIC and other ion channels (DRASIC, α -ASIC, β -ASIC). These regions can be identified from the amino acid sequence alignments disclosed herein. Highly conserved, as will be understood by those skilled in the art, means that degenerate primers can be devised which will amplify nucleic acids having the sequence characteristics of both SPASIC and the other ion channel.

For the purpose of identifying DNA encoding proteins having the currently-unique properties of SPASIC, it may be convenient to use probes or primers corresponding to all or a major part of the sequence of SEQ ID No 1, or to those parts of the sequence that are not well conserved between the different types of ASIC channel protein. Example regions include those amplified by the primers set out herein.

Preferred Examples are shown in Seq ID Nos 4 to 9. These are adapted to amplify the entire ORF (Seq ID No 4 and 5), a unique SPASIC portion for probing (Seq ID No 6 and 7) and degenerate primers having utility in finding new members of the ASIC family (Seq ID No 8 to 9).

Further suitable sequences for probes and primers of the invention will occur to those skilled in the art by alignment of the present sequence SEQ ID No 1 with the other known ASIC protein sequences. It will be realised that, where the nucleic acids of the present invention are provided in double stranded form, such probes and primers may be targeted at either strand.

Such probes, primers and oligonucleotides are provided by a further aspect of the invention and may be used in amplification reactions, or Southern or Northern blotting procedures.

5

Artificial variants (derivatives) may be prepared by those skilled in the art on the basis of the sequences provided herein, for instance by site directed or random mutagenesis, or by direct synthesis. Preferably the variant nucleic acid is generated either directly or indirectly (e.g. via one or amplification or replication steps) from an original nucleic acid having all or part of the sequence shown in Seq ID No 1.

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Thus in a further aspect of the invention there is disclosed a method of producing a derivative nucleic acid comprising the step of modifying the coding sequence of Seq ID No 1.

20

Changes to a sequence, to produce a derivative, may be by one or more of addition, insertion, deletion or substitution of one or more nucleotides in the nucleic acid, leading to the addition, insertion, deletion or substitution of one or more amino acids in the encoded polypeptide.

25

Changes may be desirable for a number of reasons, including introducing or removing the following features: restriction endonuclease sequences; codon usage; other sites which are required for post translation modification; cleavage sites in the encoded polypeptide; motifs in the encoded polypeptide for post-translational modification. Leader or other targeting sequences (e.g. membrane or golgi locating sequences) may be added to the expressed protein to determine its location following

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expression. All of these may assist in efficiently cloning and expressing an active polypeptide in recombinant form (as described below).

5 Other desirable mutation may be random (e.g. chemical) or site directed mutagenesis in order to alter the activity (e.g. specificity) or stability of the encoded polypeptide. For such methods, for example, a vectorised DNA of SEQ ID No 1 is exposed to a mutagenic material
10 such as hydroxylamine, or, in the case of SDM, a PCR reaction is carried out on that DNA using a mutagenic primer, whereby DNA is produced which encodes for a protein different in sequence to SEQ ID No 2 at a few predictable or predetermined sites respectively.

15 Particular target areas of the sequence are those which may determine any of the following: specificity of activation of the ion channel (e.g. required pH change); nature of response (e.g. transient, sustained current);
20 specificity of ion channel (e.g. relative permeability to different cations).

All of these properties can be tested, without burden, e.g. using appropriate voltage clamping techniques.

25 Preferably the amino acid sequence differs from SEQ ID No 2 only by conservative substitutions. The expression 'conservative substitutions' as used with respect to amino acids relates to the substitution of a given amino
30 acid by an amino acid having physicochemical characteristics in the same class. Thus where an amino acid in SEQ ID No 2 has a hydrophobic characterising group, a conservative substitution replaces it by another amino acid also having a hydrophobic characterising
35 group; other such classes are those where the

characterising group is hydrophilic, cationic, anionic or contains a thiol or thioether. Such substitutions are well known to those of ordinary skill in the art, i.e. see US 5380712, and are only contemplated where the resultant protein has ion channel activity.

Also included are variants having non-conservative substitutions. As is well known to those skilled in the art, substitutions to regions of a peptide which are not critical in determining its conformation may not greatly affect its activity because they do not greatly alter the peptide's three dimensional structure. In regions which are critical in determining the peptides conformation or activity such changes may confer advantageous properties on the polypeptide.

A quite different approach may include mixing or incorporating sequences from related ASIC-type genes into the SPASIC sequence. For example restriction enzyme fragments of SPASIC could be ligated together with fragments of a SPASIC homologue or even of an unrelated gene to generate recombinant variants of SPASIC (so called 'DNA shuffling' - see Crameri et al., 1998, Nature 391.).

In one aspect of the present invention, the nucleic acids of the present invention are in the form of a recombinant and preferably replicable constructs, such as vectors.

"Vector" is defined to include, inter alia, any plasmid, cosmid, phage or other vector in double or single stranded linear or circular form which may or may not be self transmissible or mobilizable, and which can transform prokaryotic or eukaryotic host either by integration into the cellular genome or exist

extrachromosomally (e.g. autonomous replicating plasmid with an origin of replication).

Specifically included are shuttle vectors by which is meant a DNA vehicle capable, naturally or by design, of replication in two different host organisms, which may be selected from actinomycetes and related species, bacteria and eucaryotic (e.g. mammalian, yeast or fungal cells).

A vector including nucleic acid according to the present invention need not include a promoter or other regulatory sequence, particularly if the vector is to be used to introduce the nucleic acid into cells for recombination into the genome.

Preferably the nucleic acid in the vector is under the control of, and operably linked to, an appropriate promoter or other regulatory elements for transcription in a host cell such as a microbial, e.g. bacterial cell. The vector may be a bi-functional expression vector which functions in multiple hosts. In the case of genomic SPASIC DNA (isolatable as described above) this may contain its own promoter or other regulatory elements and in the case of cDNA this may be under the control of an appropriate promoter or other regulatory elements for expression in the host cell.

By "promoter" is meant a sequence of nucleotides from which transcription may be initiated of DNA operably linked downstream (i.e. in the 3' direction on the sense strand of double-stranded DNA).

"Operably linked" means joined as part of the same nucleic acid molecule, suitably positioned and oriented for transcription to be initiated from the promoter.

DNA operably linked to a promoter is "under transcriptional initiation regulation" of the promoter.

Thus this aspect of the invention provides a gene construct, preferably a replicable vector, comprising a promoter operatively linked to a nucleotide sequence provided by the present invention, such as the SPASIC gene (see Seq ID NO 1) or a variant thereof.

Generally speaking, those skilled in the art are well able to construct vectors and design protocols for recombinant gene expression. Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. For further details see, for example, *Molecular Cloning: a Laboratory Manual*: 2nd edition, Sambrook et al, 1989, Cold Spring Harbor Laboratory Press.

Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis (see above discussion in respect of variants), sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Current Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds., John Wiley & Sons, 1992. The disclosures of Sambrook et al. and Ausubel et al. are incorporated herein by reference.

In one embodiment of this aspect of the present invention provides a gene construct, preferably a replicable vector, comprising an inducible promoter operatively linked to a nucleotide sequence provided by the present invention, such as Seq ID No 1.

The term "inducible" as applied to a promoter is well understood by those skilled in the art. In essence, expression under the control of an inducible promoter is "switched on" or increased in response to an applied stimulus. The nature of the stimulus varies between promoters. Some inducible promoters cause little or undetectable levels of expression (or no expression) in the absence of the appropriate stimulus. Other inducible promoters cause detectable constitutive expression in the absence of the stimulus. Whatever the level of expression is in the absence of the stimulus, expression from any inducible promoter is increased in the presence of the correct stimulus.

Particularly preferred are vectors suitable for expression of mammalian DNA, such as will occur to those skilled in the art, e.g. HSV or vaccinia vectors, or pcDNA3 shuttle vectors, e.g. as included within the lambda express system (Stratagene), which are capable of expressing heterologous protein in both bacteria and in eucaryotic cells such as COS cells. Suitable bacterial vectors will include lambda-Zap vectors such as the lambda-Zap-II vector available from Stratagene Cloning Systems. Bacterial clones containing plasmids capable of gene expression can be obtained by excising pBluescript from the lambda-Zap-II construct in the presence of a filamentous helper phage also available from Stratagene. Typical protocols are provided in the examples below, or in commercially available Stratagene kits.

A further aspect of the present invention provides cells containing, or more preferably transformed with (or transfected with) the nucleic acids of the present invention. Such cells are provided by transformation of a host cell, preferably a eucaryotic cell, e.g. a COS, CHO

or HEK 293 cell or an oocyte, preferably a Xenopus oocyte, particularly COS cells, using DNA of the invention as incorporated by recombinant DNA techniques into a vector or as directly incorporated into the cells' genomic DNA e.g. by electroporation or other such DNA integrating technique.

Such cells may be capable of expressing, or having expressed, a SPASIC protein as described hereinabove.

The cells may thus mimic, in some respects, the electrophysiological and pharmacological properties of native SPASIC-expressing cells

It is also possible to produce cells bearing the receptor protein of the invention by direct injection of RNA of the present invention, into the cells wherein it becomes translated. In the case of Xenopus oocytes it is possible to achieve the presence of functioning SPASIC protein on cell membranes within 3 days such that it is ready for electrophysiological and pharmacological study.

The foregoing discussion has been generally concerned with uses of the nucleic acids of the present invention for production of functional polypeptides, thereby increasing the SPASIC or related ion channel activity in the cell.

However the information disclosed herein may also be used to reduce the activity of SPASIC or variants thereof in cells in which it is desired to do so.

For instance down-regulation of expression of a target gene may be achieved using anti-sense technology.

In using anti-sense genes or partial gene sequences to

down-regulate gene expression, a nucleotide sequence is placed under the control of a promoter in a "reverse orientation" such that transcription yields RNA which is complementary to normal mRNA transcribed from the "sense" strand of the target gene. See, for example, Rothstein et al, 1987; Smith et al, (1988) *Nature* 334, 724-726; Zhang et al, (1992) *The Plant Cell* 4, 1575-1588, English et al., (1996) *The Plant Cell* 8, 179-188. Antisense technology is also reviewed in Bourque, (1995), *Plant Science* 105, 125-149, and Flavell, (1994) *PNAS USA* 91, 3490-3496.

"Complementary to" means that the capable of base pairing with whereby A is the complement of T (and U); G is the complement of C.

An alternative to anti-sense is to use a copy of all or part of the target gene inserted in sense, that is the same, orientation as the target gene, to achieve reduction in expression of the target gene by co-suppression.

Further options for down regulation of gene expression include the use of ribozymes, e.g. hammerhead ribozymes, which can catalyse the site-specific cleavage of RNA, such as mRNA (see e.g. Jaeger (1997) "The new world of ribozymes" *Curr Opin Struct Biol* 7:324-335, or Gibson & Shillito (1997) "Ribozymes: their functions and strategies form their use" *Mol Biotechnol* 7: 242-251.)

Methods for producing regulatory sequences will involve no burden to those skilled in the art in the light of the present disclosure. Anti-sense or sense or ribozyme based regulation may be performed using vectors as described above, and may itself be regulated by employing

an inducible promoter in an appropriate construct. For instance incorporation of this DNA into mammalian cells might be readily accomplished using vectors, e.g. such as HSV, vaccinia or adenovirus (see Principles of Gene Manipulation (1994) 5th Edit. Old and Primrose 5th Edition, Blackwell Scientific Publications).

The complete sequence corresponding to the coding sequence (in reverse orientation for anti-sense) need not be used. For example fragments of sufficient length may be used. It is a routine matter for the person skilled in the art to screen fragments of various sizes and from various parts of the coding sequence to optimise the level of anti-sense inhibition. It may be advantageous to include the initiating methionine ATG codon, and perhaps one or more nucleotides upstream of the initiating codon. A further possibility is to target a conserved sequence of a gene, e.g. a sequence that is characteristic of one or more genes, such as a regulatory sequence.

The sequence employed may be about 500 nucleotides or less, possibly about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, or about 100 nucleotides. It may be possible to use oligonucleotides of much shorter lengths, 14-23 nucleotides, although longer fragments, and generally even longer than about 500 nucleotides are preferable where possible, such as longer than about 600 nucleotides, than about 700 nucleotides, than about 800 nucleotides, than about 1000 nucleotides or more.

It may be preferable that there is complete sequence identity in the sequence used for down-regulation of expression of a target sequence, and the target sequence,

although total complementarity or similarity of sequence is not essential. One or more nucleotides may differ in the sequence used from the target gene. Thus, a sequence employed in a down-regulation of gene expression in accordance with the present invention may be a wild-type sequence (e.g. gene) selected from those available, or a variant of such a sequence.

The sequence need not include an open reading frame or specify an RNA that would be translatable. It may be preferred for there to be sufficient homology for the respective anti-sense and sense RNA molecules to hybridise. There may be down regulation of gene expression even where there is about 5%, 10%, 15% or 20% or more mismatch between the sequence used and the target gene. Effectively, the homology should be sufficient for the down-regulation of gene expression to take place.

One embodiment of this aspect of the invention employs DNA oligonucleotides, typically being of 10 to-30-bases long, conveniently about 20 bases long, optionally in degradation protected form, e.g. by being thiolated, and which conveniently have been chemically synthesized to be directed to hybridize with a part of the 5' coding region of the SPASIC mRNA. Annealing with the oligomeric DNA causes the mRNA to be degraded by activation of RNase H, or blocks the translation of the mRNA into protein. The small size of such oligomers facilitates their direct access into target cells which express the present ASIC proteins.

An alternative embodiment produces antisense RNA in vivo by inserting a tissue specific inducible or constitutively active promoter, enhancer or locus control region or element upstream of the coding region, or part

of the coding region, of DNA of SEQ ID No 1 in a construct which is then cloned into a vector. For use in mammals in therapy such a vector should be capable of infecting but not killing target cells. Convenient
5 vectors for use in this embodiment, which can target mammalian dorsal root ganglion cells are Herpes Simplex Virus (HSV) vaccinia or adenovirus derived vectors. Viral vectors for use in gene therapy are discussed by Vile (1997) Nature Biotechnology 15: 840-841. A non-viral
10 gene therapy approach is discussed by Sebestyen et al (1998) Nature Biotechnology 16: 80-85. The use of a variety of gene therapy delivery systems (including HSV VP22) is discussed by Fernandez & Baylay (1998) Nature Biotechnology 16: 418-420 and references therein.

15 Where the antisense down-regulating DNA or RNA is provided in dorsal root ganglia cells it potentially inhibits the pain response by actually decreasing the number of SPASIC channels on the surface of sensory
20 cells. Where the antisense down-regulating DNA or RNA is provided in the central nervous system, it alters neurotransmitter release by actually decreasing the number of SPASIC channels on the surface of neurons.

25 Thus a nucleotide sequence which is complementary to any of the coding-nucleic acids discussed in relation to earlier aspects of the invention forms one part of the present invention.

30 The invention further provides a method of influencing the electrophysiological and pharmacological properties of a cell, said method comprising the step of causing or allowing expression of a heterologous nucleic acid
35 sequence as discussed above within that cells, which may optionally be within an organism.

5 The present invention further provides the use of the nucleotide sequence of Seq ID No 1, or its complement, or a variant of either for down-regulation of gene expression, particularly down-regulation of expression of an ion channel-encoding gene, more preferably a cation channel, most preferably SPASIC.

10 In a further aspect of the present invention there is provided nucleic acid (e.g. antisense DNA) of the present invention for use in gene therapy, or for use in the preparation of medicaments for use in gene therapy.

15 In a further aspect of the present invention there is disclosed an organism, preferably a non-human mammal, comprising cells in which the activity of SPASIC or a variant thereof have been altered, preferably impaired, by use of the methods and materials discussed above. Particularly preferred is a rodent e.g. murine organism. Methods of producing 'knock out' mammals in which
20 specific gene activities have been impaired are now well known to those skilled in the art - see e.g. Boerrigter et al (1995) Nature 377: 657-659, or Gossen and Vijk (1993) Trends Genet 9: 27-31.

25 In a further aspect of the present invention there is provided use of a protein, a transformed or transfected cell, or a transgenic organism as described above, for identifying a substance as having ion-channel modulating activity.

30 Such substances may, for instance, act as agonist, partial agonist or antagonist.

35 Thus the protein or cell may be used in a method comprising exposing the protein (e.g which is associated

with a membrane, for instance of a liposome) or cell surface to a solution of the substance such as to allow interaction between the substance and the SPASIC (or variant SPASIC) protein in the membrane and then measuring the electrophysiological response of the cell or membrane to this interaction. This measurement may optionally be compared with a reference figure.

Typically the response may be measured by use of a microelectrode technique accompanied by such measurement strategies as voltage clamping of the cell whereby activation of ion channels may be identified by inward or outward current flow as detected using the microelectrodes. ^{22}Na , ^{86}Rb , ^{45}Ca radiolabeled cations or ^{14}C or ^3H guanidine may be used to assess such ion flux; a sodium, calcium or potassium ion sensitive dye (such as Fura-2, or indo) may be used to monitor ion passage through the receptor ion channel, or a potential sensitive dye may be used to monitor potential changes, e.g. such as in depolarization.

Agonists and partial agonists may be identified by their relative efficacy as compared to extracellular acid pH or other known agonists in activating the receptor or, in the case of partial agonists and antagonists, by their ability to block the activation caused by a given acidity, for example pH 6, or other known agonists.

Such substances will have potential as analgesics or other neuromodulatory agents, and as such form a further aspect of the present invention, optionally in the form medicaments (e.g. compositions comprising a pharmaceutically acceptable carrier or filler).

Purified SPASIC protein, or a variant thereof, e.g.

produced recombinantly by expression from encoding nucleic acid therefor, may be used to raise antibodies employing techniques which are standard in the art.

5 Antibodies and polypeptides comprising antigen-binding fragments of antibodies may be used (*inter alia*) as antagonists or inhibitors, or for use in identifying homologues.

10 Methods of producing antibodies include immunising a mammal with the protein or a fragment thereof. Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and might be screened, preferably using binding of antibody
15 to antigen of interest.

For instance, Western blotting techniques or immunoprecipitation may be used (Armitage et al, 1992, Nature 357: 80-82). Antibodies may be polyclonal or
20 monoclonal.

Antibodies may be modified in a number of ways. Indeed the term "antibody" should be construed as covering any specific binding substance having a binding domain with
25 the required specificity. Thus, this term covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including any polypeptide comprising an immunoglobulin binding domain, whether natural or synthetic. Chimaeric molecules comprising an
30 immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore included. Cloning and expression of Chimaeric antibodies are described in EP-A-0120694 and EP-A-0125023. It has been shown that fragments of a whole antibody can perform the function of
35 binding antigens. Examples of binding fragments are (i)

the Fab fragment consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward, E.S. et al., Nature 341, 544-546 (1989) which consists of a VH domain; (v) isolated CDR regions; (vi) F(ab')₂ fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird et al, Science, 242, 423-426, 1988; Huston et al, PNAS USA, 85, 5879-5883, 1988); (viii) bispecific single chain Fv dimers (PCT/US92/09965) and (ix) "diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804; P Holliger et al Proc. Natl. Acad. Sci. USA 90 6444-6448, 1993).

The present invention will now be described by way of illustration only by reference to the following non-limiting Figures, Sequence Listing and Examples. Further embodiments of the invention falling into the scope of the claims provided herewith will occur to those of ordinary skill in the art in the light of these.

Figures

Fig 1: Aligned amino acid sequences of SPASIC (protein of present invention), DRASIC (see Waldmann and Lazdunski 1998, Current Biology 8: 418-424); ASIC-alpha (also referred to as 'ASIC' - see also Waldmann and Lazdunski *supra*); ASIC-beta (GB 9718365.1 - unpublished at the filing date of the present application). Trans-membrane helices (TM1/TM2) are marked.

Fig 2: Shows a time-course the inward current (in pA) evoked by application of low pH (about 5) to a COS cell expressing SPASIC. The characteristic sustained inward current is clearly visible.

5

Sequence listings

The listing provided herewith gives the DNA and amino acid sequences of the following examples of the invention. Numbering of primers is with respect to Seq ID Nos 1 and 2 as appropriate.

10

SEQ ID No 1: cDNA corresponding to SPASIC cDNA as derived from Rattus norvegicus dorsal root ganglia cells.

15

SEQ ID No 2: Amino acid sequence of SPASIC protein from Rattus norvegicus dorsal root ganglia cells.

20

SEQ ID No 3: Amino acid sequence of ASIC protein of the prior art.

SEQ ID No 4: Primer for use with that of SEQ ID No 5 PCR screening (253-277)

25

SEQ ID No 5: Primer for use with that of SEQ ID No 4 in PCR screening (reverse 1955-1980)

SEQ ID No 6: Primer for use with that of SEQ ID No 7 in PCR screening (419-438)

30

SEQ ID No 7: Primer for use with that of SEQ ID No 6 in PCR screening (reverse 984-1008)

35

SEQ ID No 8: Primer for use with that of SEQ ID No 9 in

degenerate PCR screening (corresponds to amino acid sequence RYG/AKEI/LSM - numbers 379-386).

SEQ ID No 9: Primer for use with that of SEQ ID No 8 in PCR screening (corresponds, in reverse, to amino acid sequence GLFIGASI/L in the TM2 region of Figure 1 - numbers 438-445).

Examples

Example 1: Isolation of SPASIC

The rat ASIC-beta clone (Patent application GB 9718365.1) was used to probe a cDNA library. 10^6 clones from a size fractionated (2-4kb) DRG cDNA library were amplified on HyBond N+ filters (Amersham) prior to hybridisation (Woo et al. PNAS (1978) 75, 3688) 32-P dATP random-primed ASIC-beta EcoRI probe (position 1-2087) was used to probe the library. The filters were hybridised at low stringency in 6 x SSC, 0.5% SDS, 5 x Denhardt's solution, with 200 micrograms/ml boiled salmon sperm DNA at 53°C. The filters were washed briefly in 4 x SSC at room temperature, then twice in 2 x SSC with 0.5% SDS at 55°C for 20 minutes. The filters were autoradiographed for 3 days on Kodak X-Omat films.

The ASIC-beta EcoRI probe (position 1-2087) was also used for high stringency screening where the filters were given a final wash in 0.1 x SSC, 0.5% SDS at 68°C (Chen et al. 1995 Nature 377, 428-432). Plaques that hybridised at low stringency but not at high stringency were selected for secondary screening, followed by cross-hybridisation. Full length SPASIC clones were isolated in this way.

Example 2: Preparation of cDNA from *E. coli*

Bluescript plasmid DNA encoding for the protein of the invention was purified from the *E. coli* DNA by solvent
5 extraction, Magic minipreps or Caesium chloride centrifugation, and digested with endonucleases EcoRI and XhoI targeted at the 3'-polylinker downstream of the cDNA and the site of the vector bacteriophage polymerase.

Example 3: Homology Cloning of other mammalian SPASIC using sequence derived from the rat cDNA clone.

Dorsal root ganglia or central nervous system tissue are isolated from available human or other mammalian source
15 tissue in accordance with methods well known to those skilled in the art. RNA is extracted from the isolated tissue by extraction in guanidine or other chaotropic agents, followed by solvent extraction using phenol, phenol/chloroform and precipitation using isopropanol and ethanol (see Chomczynski & Sacchi, 1987, Anal Biochem
20 162: 156-159).

The isolated RNA is Northern blotted with probes derived from the SEQ ID No 1 to identify transcript size of
25 candidate human clones. Messenger RNA from the RNA pool is extracted using oligo-dT cellulose or poly-U sepharose chromatography and cDNA is constructed from this RNA using reverse transcriptase (SuperScript) and DNA ligase. Double-stranded DNA is constructed and a directional
30 cDNA library generated using lambda zap, lambda express, pcDNA, or other suitable vectors.

The 3' untranslated region of the SPASIC clone is sub-cloned into a plasmid vector (Bluescript or similar)
35 followed by cutting out the insert and isolating it on

agarose gels. cRNA clones are labelled by *in vitro* transcription with SP6, T3 or T7 polymerases and ³²P or DIG labelled nucleotides. Alternatively the insert is radiolabelled by random prime or nick translation with ³²P or DIG-labelled nucleotides.

The cDNA library is screened by moderate stringency hybridisation to 50-60°C 5 x SSC, using radiolabelled or other labelled DNA or cRNA probes derived from the 3'-UTR of the DNA/RNA sequence. Alternatively, other regions of the protein may be used. Resulting clones are plaque purified and their insert sized examined. Cross hybridisation of the clones follows with isolation of individual distinct clones. Clones that contain inserts that correspond approximately to the size of human mRNAs determined by Northern blotting are isolated. cRNA is generated from the selected clones and injected into Xenopus oocytes or COS (up to 50nls, 1mg/ml) and using twin electrode voltage clamp studies, the expression of functional proton-gated channels investigated.

Functionally active channel clones have their insert DNA sequenced and this DNA is recloned into a selectable shuttle vector (e.g. pcDNA neo- Invitrogen) to generate transiently or permanently transfected cell lines (COS cells, CHO cells, 293 cells etc.) expressing functional human ASIC protein clones. These cell lines may then be used with either ²²Na, ⁸⁶Rb, ⁴⁵Ca ion flux measurement, electrophysiology or Na or Ca-sensitive dye techniques to provide a high-throughput screen for channel agonist or antagonist candidate compounds.

Example 4: PCR cloning of other mammalian SPASIC using sequence derived from the rat cDNA clone.

Dorsal root ganglia and spinal cord are are isolated from available human or other mammalian tissue material. RNA is extracted from the isolated tissue by extraction in guanidine or other chaotropic agents, followed by solvent extraction using phenol, phenol/chloroform and precipitation using isopropanol and ethanol. Random primers and reverse transcriptase are used to generate cDNA from the extracted human RNA using either total or poly A+ RNA.

Degenerate PCR primers derived from conserved regions of SEQ ID No-1 are used to amplify the cDNA using the polymerase chain reaction; e.g. using degenerate primer sequences SEQ ID No 4 and 5, 6 and 7 and 8 and 9. The products of the PCR reaction are separated on agarose gels and examined with products of the approximate predicted size being extracted and cloned into a pGemT vector. The clones are sequenced and the sequences examined for similarity with that of SEQ ID No 1. Candidate PCR fractions are used to screen a human cDNA DRG library as described in Example 2.

Example 5: In vitro expression of proton gated ASIC channels.

Plasmids encoding SPASIC cDNA are cut with restriction enzymes XhoI and EcoRI. The SPASIC insert, which comprises the protein encoding regions, is isolated from low melting agarose gel, and subcloned into pTracer-CMV and pgwl vectors respectively between EcoRI and XhoI restriction sites. The orientation of these constructs is checked by sequencing.

Shuttle vectors eg. pTracer-CMV or pgwl containing SPASIC were purified from maxipreps. These vectors were used to

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express proton-gated channels by transfecting permanent cell line COS cells. Cultured COS cells from a 100 mm petri dish (80-90% confluent) were trypsinised and resuspended in 350 microlitres of ice cold HEBS buffer. 20-30 micrograms of plasmids of interest were dissolved with 150 microlitres of HEBS buffer, then mixed with the COS cell suspension in an electroporation cuvette and kept on ice to cool for 5 minutes. Meanwhile, the electroporator (Invitrogen) was set up at 250 microFarad, and charged for 3 minutes at 330V, 25mA, and 25W. The cuvette was flicked to resuspend cells and electroporation effected.

After transfection by electroporation, COS cells were seeded in low density in-30mm petri dish and cultured with 2ml MEM/10% FCS at 37°C for 2-3 days. The transfected cells were washed with buffer (in mM NaCl 146, KCl 5, CaCl₂ 2, MgCl₂ 1, Glucose 10, HEPES 10 at various pHs) and using the whole cell configuration of the patch-clamp technique, currents evoked by perfusing with low pH buffer (pH 5) were measured at a holding potential of -60mV, with a 20 second perfusion duration of pH 5 buffer (see Figure 2). Compounds to be assessed as agonists, partial agonists or antagonists of the ASIC channels were bath applied and inward current used as measurement of the activation or block of channels encoded by the transfected vectors.

Example 6: Screening for ion channel modulating agents

Permanently or transiently transfected COS cell lines expressing SPASIC are loaded with C-14 guanidine (10 microcuries per ml) in multiwell plates for 1 hour at 37°C in DHEM growth medium containing 10% calf serum, washed in HEPES buffered saline (pH 7.4) 4 times, and

rates of radioactivity released measured in the presence and absence of a low pH stimulus (pH 5.5 Hepes buffered saline), with or without the addition of compounds that may show agonist, partial agonist or antagonist activity
5 (Wood et al, 1989 J Neurochem 53: 1203-1211).

Alternatively, permanently or transiently transfected COS cell lines in multiwell plates expressing SPASIC are loaded with potential-sensitive carbocyanine dyes and
10 the fluorescence emission spectral response is observed following application of a low pH stimulus (pH 5.5 Hepes buffered saline). The response in the presence and absence of the putative activating compound is compared
15 (see Am J Physiol 266, C37 1994).

09701347.012301
105210.44060

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Claims

- 1 An acid sensitive cation channel protein which is a SPASIC protein comprising amino acid sequence Seq ID No 2.
- 5 2 A SPASIC variant protein which
(i) has ion channel activity, and
(ii) comprises an amino acid sequence having at least 80% sequence identity with the full length sequence shown in Seq ID No 2.
- 10 3 A SPASIC variant protein as claimed in claim 2 which is a fragment of the SPASIC protein of claim 1.
- 15 4 A SPASIC variant protein as claimed in claim 2 which is capable of reversibly mediating (i) a rapid, and (ii) a sustained cation current.
- 20 5 An isolated nucleic acid comprising a polynucleotide sequence encoding the protein of any one of the preceding claims.
- 25 6 A nucleic acid as claimed in claim 5 wherein the polynucleotide sequence comprises bases 292-1909 of Seq ID No 1 or is degeneratively equivalent thereto.
- 7 A nucleic acid as claimed in claim 5 which encodes the SPASIC variant of claim 3.
- 30 8 A nucleic acid as claimed in claim 7 which is capable of hybridising with the complement of the nucleic acid of claim 6 under high stringency conditions, being 0.1 x SSC, 0.5% SDS at 68°C.
- 35 9 A nucleic acid as claimed in claim 7 or claim 8 which is an allelic variant.

10 A nucleic acid which is the complement of the nucleic acid of any one of claims 5 to 9.

5 11 A nucleic acid molecule for use as a probe, which molecule comprises a contiguous polynucleotide sequence of at least 30, 50 or 100 bases found in Seq ID No 1, or a sequence degeneratively equivalent thereto, or the complement of either.

10 12 A nucleic acid molecule for use as a primer, which molecule comprises a contiguous polynucleotide sequence of at least 16, 18, 21 or 24 bases found in Seq ID No 1, or a sequence degeneratively equivalent thereto, or the complement of either.

15 13 A nucleic acid molecule as claimed in claim 12 wherein the contiguous polynucleotide sequence is one which is highly conserved between SPASIC and one or more other ion channel proteins selected from: DRASIC ; α -ASIC ; β -ASIC.

20 14 A nucleic acid molecule as claimed in claim 12 wherein the contiguous polynucleotide sequence is one which is not present in any of DRASIC ; α -ASIC ; β -ASIC.

15 A nucleic acid molecule for use as a primer, which molecule is selected from: Seq ID No 4, 5, 6, 7, 8, or 9.

30 16 A method of identifying and/or cloning the nucleic acid according to any one of claims 7 to 9, which method employs the nucleic acid molecule of any one of claims 11 to 15.

35 17 A method as claimed in claim 16 comprising the steps of:

(a) providing a preparation of nucleic acid,

(b) providing the nucleic acid molecule of any one of claims 11 to 15,

(c) contacting nucleic acid in said preparation with said nucleic acid molecule under conditions for hybridisation,

5 (d) identifying said SPASIC variant if present by its hybridisation with said nucleic acid molecule.

18 A method as claimed in claim 16 comprising the steps of:

10 (a) providing a preparation of nucleic acid,

(b) providing a pair of nucleic acid molecule primers suitable for PCR, at least one said primers being the nucleic acid molecule of any one of claims 12 to 15,

15 (c) contacting nucleic acid in said preparation with said primers under conditions for performance of PCR,

(d) performing PCR and determining the presence or absence of an amplified PCR product.

19 A method as claimed in claim 17 or claim 18 wherein
20 the nucleic acid preparation is derived from dorsal root ganglia or spinal cord.

20 A method of producing a derivative nucleic acid acid according to claim 7 or claim 8, which method comprises
25 the step of modifying a nucleic acid comprising Seq ID No 1.

21 A recombinant vector comprising the nucleic acid of any one of claims 5 to 10.

30

22 A vector as claimed in claim 21 wherein the nucleic acid is operably linked to a promoter or other regulatory element for transcription in a host cell

35 23 A vector as claimed in claim 22 which is suitable for expression of the nucleic acid in a eucaryotic cell.

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24 A host cell containing a heterologous nucleic acid of any one of claims 5 to 10.

5 25 A host cell transformed with the vector of any one of claims 21 to 23.

10 26 A host cell as claimed in claim 24 or claim 25 which is selected from: a COS, CHO or HEK 293 cell or an oocyte.

15 27 A host cell comprising a heterologous protein of any one of claims 1 to 4.

20 28 A method of influencing the electrophysiological and/or pharmacological properties of a cell, said method comprising the step of causing or allowing expression of a heterologous nucleic acid of any one of claims 5 to 10 within the cell.

25 29 A method as claimed in claim 28 for increasing the ion channel activity in the cell.

30 30 A method as claimed in claim 28 for reducing the ion channel activity in the cell.

35 31 A method as claimed in claim 30 comprising the use of any of (i) all or part of the nucleic acid of claim 10 to reduce the activity by an anti-sense mechanism, (ii) part of the nucleic acid of any one of claims 5 to 9 to reduce activity by co-suppression, or (iii) use of a ribozyme specific for a nucleic acid of any one of claims 5 to 9.

32 A transgenic non-human mammal, comprising a cell of any one of claims 24 to 27 and/or a cell in which the electrophysiological and/or pharmacological properties has been altered in accordance with the method of any one

of claims 28 to 31.

33 A method for identifying a substance having ion-channel modulating activity, the method comprising the use of any of (i) the protein of any one of claims 1 to 4, (ii) a cell of any one of claims 24 to 27, (iii) a cell in which the electrophysiological and/or pharmacological properties has been altered in accordance with the method of any one of claims 28 to 31 (iv) the transgenic organism of claim 32.

34 A method as claimed in claim 33 comprising the steps of:

(i) exposing the protein of any one of claims 1 to 4, which is associated with a membrane or cell surface, to a solution of the substance such as to allow interaction between the substance and the protein,
(ii) measuring the electrophysiological response of the cell or membrane to this interaction.

35 A method as claim in claim 33 or claim 34 for screening for potential analgesics; neuromodulatory agents; anti-inflammatory agents; agents that regulate neurotransmitter release or neuronal excitability.

36 A method of influencing the electrophysiological and/or pharmacological properties of a cell, said method comprising the step of modulating the activity of the protein of any one of claims 1 to 4.

37 A polypeptide comprising an antigen-binding site of an antibody capable of specifically binding the protein of any one of claims 1 to 4.

38 Nucleic acid of any one of claims 5 to 10, or the vector of any one of claims 21 to 23, for use in gene therapy, or for use in the preparation of a medicament

for use in gene therapy.

- 5 39 The nucleic acid or vector of claim 39 wherein the therapy comprises the step of inhibiting a pain response and/or altering neurotransmitter release.

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Fig. 1A

SPASIC M P I E I V C K I K F A E E D A K P
 DRASIC M K P R S G L E E A Q R R Q A S D I
 α -ASIC M E L K T E E E E V G G V Q P V S I
 β -ASIC M E A G S E L D E....G D D S P R D L

SPASIC K E K E A G D E Q S L L G A A Q G P
 DRASIC R V F A S S C T M H G - - H I F.....
 α -ASIC Q A F A S S S T L H G - A H I F.....
 β -ASIC V A F A N S C T L H G A S H V F.....

SPASIC A A P R D L A T F A S T S T L H G L
 DRASIC
 α -ASIC
 β -ASIC

SPASIC G R A C G P G P H G L R R T L W V L
 DRASIC- - - G L T - - - G - - A T
 α -ASICS Y E R L S - K - A - - A -
 β -ASICV E - G P - P - Q A - - A V

SPASIC A L L T S L A A F L Y Q A A S L A R
 DRASIC - V - L - - - - - - - V - E R V -
 α -ASIC C F - G - - - V L - C V C T E R V Q
 β -ASIC - F V I A - G - - - C - V G D R V A

TM1

SPASIC G Y L T R P H L V A M D P A A P A P
 DRASIC Y - G E F H - K T T L - E R E S H Q
 α -ASIC Y - F C Y H - V T K L - E V - A S Q
 β -ASIC Y - - S Y - - V T L L - E V - T S E

SPASIC V A G F P A V T L C N I N R F R H S
 DRASIC L T.....- - - - - - - - P L - R -
 α -ASIC L T.....- - - - - - - L - E - - F -
 β -ASIC L V.....- - - - - F - - T - A V - L -

SPASIC A L S D A D I F H L A N L T G L P P
 DRASIC R - T P N - L H W A G T A L L G L D
 α -ASIC Q V - K N - L Y - A G E - L A - L N
 β -ASIC Q - - Y P - L L Y - - P M L - - D E

SPASIC K D R D.....G H R A A G L
 DRASIC P A E H A A Y L R A L - Q P P - P P
 α -ASIC N R Y E I P D T Q M A D E K Q L E I
 β -ASIC S - D P G V P L A P P - P E - F S G

Fig. 1B

SPASIC	R	Y	P	E	P	D	M	V										
DRASIC	G	F	M	P	S	P	T	F	D	M	A	Q	L					
α-ASIC	L	Q	D	K	A	N	F	R	S	F	K	P	K	P	F	N	-	R	
β-ASIC	E	P	F	N	L	H	R											
SPASIC	D	I	L	N	R	T	G	H	Q	L	A	D	M	L	K	S	C	N	
DRASIC	Y	A	-	A	-	-	S	-	E	-	-	-	L	D	-	R		
α-ASIC	E	F	Y	D	-	A	-	-	D	I	R	-	-	-	L	-	-	H	
β-ASIC	...F	Y	N	-	S	C	-	R	-	E	-	-	-	L	Y	-	S		
SPASIC	F	S	G	H	H	C	S	A	S	N	F	S	V	V	Y	T	R	Y	
DRASIC	Y	R	-	Q	P	-	G	P	E	-	-	T	-	I	F	-	-	M	
α-ASIC	-	R	-	E	A	-	-	-	E	D	-	K	-	-	F	-	-	-	
β-ASIC	Y	C	-	G	P	-	G	P	H	-	-	-	-	-	F	-	-	-	
SPASIC	G	K	C	Y	T	F	N	A	D	P	Q	S	S	L	P	S		
DRASIC	-	Q	-	-	-	-	-	S	G	A	H	G	A	E	L	L	T	T	
α-ASIC	-	-	-	-	-	-	-	S	G	Q	D	G	R	P	R	L	K	T	
β-ASIC	-	-	-	-	-	-	-	S	G	Q	D	G	R	P	R	L	K	T	
SPASIC	R	A	G	G	M	G	S	G	L	E	I	M	L	D	I	Q	Q	E	
DRASIC	P	K	-	-	A	-	N	-	-	-	-	-	-	-	V	-	-	-	
α-ASIC	M	K	-	-	T	-	N	-	-	-	-	-	-	-	-	-	-	D	
β-ASIC	M	K	-	-	T	-	N	-	-	-	-	-	-	-	-	-	-	D	
SPASIC	E	Y	L	P	I	W	R	E	T	N	E	T	S	F	E	A	G	I	
DRASIC	-	-	-	-	-	-	K	D	M	E	-	-	P	-	-	V	-	-	
α-ASIC	-	-	-	-	V	-	G	-	-	D	-	-	-	-	-	-	-	-	
β-ASIC	-	-	-	-	V	-	G	-	-	D	-	-	-	-	-	-	-	-	
SPASIC	R	V	Q	I	H	S	Q	E	E	P	P	Y	I	H	Q	L	G	F	
DRASIC	-	-	-	-	-	-	D	-	-	-	-	A	-	D	-	-	-	-	
α-ASIC	K	-	-	-	-	-	D	-	-	-	-	F	-	D	-	-	-	-	
β-ASIC	K	-	-	-	-	-	D	-	-	-	-	F	-	D	-	-	-	-	
SPASIC	G	V	S	P	G	F	Q	T	F	V	S	C	Q	E	Q	R	L	T	
DRASIC	-	A	A	-	-	H	-	-	-	-	-	-	-	Q	-	Q	-	S	
α-ASIC	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	
β-ASIC	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	
SPASIC	Y	L	P	Q	P	W	G	N	C	R	A	E	S	K	L	R	E	
DRASIC	F	-	-	P	-	-	-	D	-	N	T	-	-	-	-	D	P	
α-ASIC	-	-	-	S	-	-	-	T	-	N	-	V	T	M	D	S	...	
β-ASIC	-	-	-	S	-	-	-	T	-	N	-	V	T	M	D	S	...	
SPASIC	P	E	L															
DRASIC	D	D	F	D	P	E	P	S	D	P	L	G	S	P	R	P		
α-ASIC	D	F	F	D														
β-ASIC	D	F	F	D														

Fig. 1C

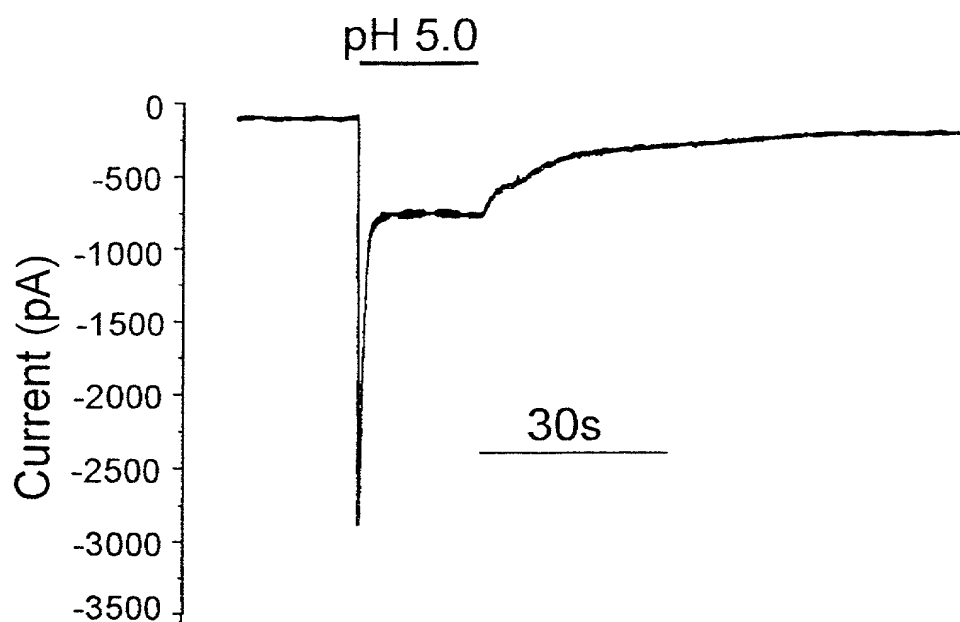
SPASIC	Q	G	Y	S	A	Y	S	V	S	A	C	R	L	R	C	E	K	E
DRASIC	R	P	S	P	P	-	-	L	I	G	-	-	-	A	-	-	S	R
α -ASIC	S	-	-	I	T	-	-	-	I	D	-	-	T	R			
β -ASIC	S	-	-	I	T	-	-	-	I	D	-	-	T	R			
SPASIC	A	V	L	Q	R	C	H	C	R	M	V	H	M	P	G	N	E	T
DRASIC	Y	-	A	R	K	-	G	-	-	-	M	-	-	-	-	-	S	P
α -ASIC	Y	L	V	E	N	-	N	-	-	-	-	-	-	-	-	D	A	P
β -ASIC	Y	L	V	E	N	-	N	-	-	-	-	-	-	-	-	D	A	P
SPASIC	I	C	P	P	N	I	Y	I	E	C	A	D	H	T	L	D	S	L
DRASIC	V	-	S	-	Q	Q	-	K	D	-	-	S	P	A	-	-	A	M
α -ASIC	Y	-	T	-	E	Q	-	K	-	-	-	-	P	A	-	-	F	-
β -ASIC	Y	-	T	-	E	Q	-	K	-	-	-	-	P	A	-	-	F	-
SPASIC	G	G	G	S	E	G	P	C	F	C	P	T	P	C	N	L	T	R
DRASIC	L	R	K	D	T	-	V	-	-	N	-	-	A	T	-	-	
α -ASIC	V	E	K	D	Q	E	Y	-	V	-	E	M	-	-	-	-	-	
β -ASIC	V	E	K	D	Q	E	Y	-	V	-	E	M	-	-	-	-	-	
SPASIC	Y	G	K	E	I	S	M	V	K	I	P	N	R	G	S	A	R	Y
DRASIC	-	A	-	-	L	-	-	-	R	-	-	S	-	A	-	-	-	
α -ASIC	-	-	-	-	L	-	-	-	-	-	-	S	K	A	-	-	K	-
β -ASIC	-	-	-	-	L	-	-	-	-	-	-	S	K	A	-	-	K	-
SPASIC	L	A	R	K	Y	N	R	N	E	T	Y	I	R	E	N	F	L	V
DRASIC	-	-	-	-	-	-	-	S	-	S	-	-	T	-	-	V	-	
α -ASIC	-	-	K	-	F	-	K	S	-	Q	-	-	G	-	-	I	-	
β -ASIC	-	-	K	-	F	-	K	S	-	Q	-	-	G	-	-	I	-	
SPASIC	L	D	V	F	F	E	A	L	T	S	E	A	M	E	Q	R	A	A
DRASIC	-	-	I	-	-	-	-	-	N	Y	-	-	V	-	-	K	-	
α -ASIC	-	-	I	-	-	-	V	-	N	Y	-	T	I	-	-	K	K	-
β -ASIC	-	-	I	-	-	-	V	-	N	Y	-	T	I	-	-	K	K	-
SPASIC	Y	G	L	S	A	L	L	G	D	L	G	G	Q	M	G	L	F	I
DRASIC	-	E	V	-	E	-	-	-	-	I	-	-	-	-	-	-	-	
α -ASIC	-	E	I	A	G	-	-	-	-	I	-	-	-	-	-	-	-	
β -ASIC	-	E	I	A	G	-	-	-	-	I	-	-	-	-	-	-	-	
SPASIC	G	A	S	I	L	T	L	L	E	I	L	D	Y	I	Y	E	V	S
DRASIC	-	-	-	L	-	-	I	-	-	-	-	-	-	L	C	-	-	F
α -ASIC	-	-	-	-	-	-	V	-	-	L	F	-	-	A	-	-	-	I
β -ASIC	-	-	-	-	-	-	V	-	-	L	F	-	-	A	-	-	-	I
TM2																		
SPASIC	W	D	R	L	K	R	V	W	R	R	P	K	T	P	L	R
DRASIC	Q	-	-	V	L	G	Y	F	-	N	-	-	S	A	Q	K	-
α -ASIC	K	H	-	-	C	R	-	G	K	C	Q	-	E	A	K	-	

Fig. 1D

β -ASIC	K	H	-	-	C	R	-	G	K	C	Q.....-	E	A	K	-
SPASIC	T	S	T	G	G	I	S	T	L	G	L	Q	E	L	K
DRASIC	...	-	G	N	T	L	L	Q	E	E	-	N	G	H	R
α -ASIC	S	-	A	D	K	G	V	A	-	S	-	D	D	V	-
β -ASIC	S	-	A	D	K	G	V	A	-	S	-	D	D	V	-
SPASIC	P	C	P	N	R	G	R	A	E	G	G	G	A	S	N
DRASIC	-	H	L	S	L	-	P	R	P	P	T	T	P	C	A
α -ASIC	-	-	E	S	L	R	G	H	P	A	-	M	T	Y	A
β -ASIC	-	-	E	S	L	R	G	H	P	A	-	M	T	Y	A
SPASIC	N	H	H	H	P	H	G	P	P	G	S	L	F	E	N
DRASIC	T	L	S	A	S	-	R	T	C	Y	L	V	T	R	L
α -ASIC	L	P	-	-	-	A	R.....-T	-	-	D	-	T	-	
β -ASIC	L	P	-	-	-	A	R.....-T	-	-	D	-	T	-	

Fig. 2

SPASIC Expressed in COS-7 Cells



RULE 53 (37 C.F.R. 1.63)
INVENTORS DECLARATION FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

As a below named inventor, I hereby declare that my residence, mailing address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

ION CHANNELS

the specification of which (check applicable box(es)):

☐ is attached hereto
☐ was filed on _____ as U.S. Application Serial No. _____ (Atty Dkt. No. 620-123)
☒ was filed as PCT International application No. PCT/GB99/01743 on 3 June 1999
and (if applicable to U.S. or PCT application) was amended on 4 September 2000 And 14 September 2000

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose to the Patent Office all information known to me to be material to patentability as defined in 37 C.F.R. 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed or, if no priority is claimed, before the filing date of this application:

Priority Foreign Application(s):

Application Number	Country	Day/Month/Year Filed
9811965.4	GREAT BRITAIN	3 June 1998

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

Application Number	Date/Month/Year Filed
--------------------	-----------------------

I hereby claim the benefit under 35 U.S.C. 120/365 of all prior United States and PCT International applications listed above or below:

Prior U.S./PCT Application(s):

Application Serial No.	Day/Month/Year Filed	Status: patented pending, abandoned
PCT/GB99/01743	3 June 1999	

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. And on behalf of the owner(s) hereof, I hereby appoint NIXON & VANDERHYE P.C., 1100 North Glebe Rd., 3rd Floor, Arlington, VA 22201-4714, telephone number (703) 816-4000 (to whom all communications are to be directed), and the following attorneys-in-fact (of the same address) individually and collectively owner's/owners' attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent: Arthur R. Crawford, 25327; Larry S. Nixon, 25640; Robert A. Vanderhye, 27076; James T. Hosmer, 30184; Robert W. Farris, 31358; Richard G. Beeha, 22770; Mark E. Nusbaum, 32348; Michael J. Keenan, 32106; Bryan H. Davidson, 30251; Stanley C. Spooner, 27393; Leonard C. Mitchard, 29009; Duane M. Byers, 33363; Jeffrey H. Nelson, 30481; John R. Lastova, 33149; H. Warren Burnam, Jr., 29366; Thomas E. Byrne, 32205; Mary J. Wilson, 32955; J. Scott Davidson, 33489; Alan M. Kagen, 36176; Robert A. Molan, 29834; B. J. Sadoff, 36663; James O. Berquist, 34776; Updeap S. Gill, 37334; Michael J. Shea, 34725; Donald L. Jackson, 41090; Michelle N. Lester, 32331; Frank P. Prestia, 19928; Joseph S. Prestia, 35329; Joseph A. Rhoads, 37615; Raymond Y. Mah, 41428. I also authorize Nixon & Vanderhye to delete any attorney names/numbers no longer with the firm and to act and rely solely on instructions directly communicated from the person, assignee, attorney, firm, or other organization sending instructions to Nixon & Vanderhye on behalf of the owner(s).

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FOR ADDITIONAL INVENTORS, check box ☐ and attach sheet with same information and signature and date for each.

09701747-012901

SEQUENCE LISTINGS

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 2623 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

10 (iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Rattus norvegicus
(F) TISSUE TYPE: Dorsal root ganglion

(ix) FEATURE:

- 15 (A) NAME/KEY: CDS
(B) LOCATION: 292..1909

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

spasic Length: 2622

20 1 AGTGACAGCT GTGCGGGTGC TGATAAGGGA AGCCACAAGG AGACGATCGA
51 GGAGAGAGAC AAGCGGCAGC AGAGGCAGCA GCGACAGATG CAGCGCCGGG
101 GCTGCGGAGC TGCTGGGAGT GGGAGTGACG CCCCCACCTC GGGCCCCCAC
25 151 CCTGTCCCCA TCCTCCTCCT GGTGCCCCTG AGTTTAGAAG AGCAGCCGCT
201 GCCACCACCA CCACTCCGGA GGGCACCAGG GCTGCTGTCC AGGGAAGGAC
30 251 AGTAGCAGTG AGGCTCTGGC CAGTCCCAGC AGCCGGGGAC AGATGCCGAT
301 CGAGATTGTG TGCAAAATCA AATTTGCTGA GGAGGATGCA AAACCCAAGG
351 AGAAGGAGGC AGGGGATGAG CAGAGCCTCC TGGGGGCTGC TCAGGGGCCA
35 401 GCAGCCCCTC GGGACCTGGC TACCTTTGCC AGCACCAGTA CTCTGCATGG

451 GCTGGGCCCG GCCTGTGGCC CAGGCCCCCA TGGACTGCGC AGAACCCTGT
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5 551 AGCCTGGCCA GGGGCTACCT GACCCGGCCT CACCTGGTAG CCATGGACCC
601 TGCTGCCCCA GCCCCAGTGG CGGGCTTTCC GGCTGTCACC CTCTGCAACA
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901 CACCTTCAAT GCAGATCCTC AGAGTTCCT GCCCAGCAGG GCAGGCGGCA
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25 1051 GCAGATCCAC AGCCAGGAGG AGCCTCCCTA CATCCACCAG CTGGGGTTTCG
1101 GTGTGTCCCC AGGCTTCCAG ACTTTTGTGT CCTGCCAGGA ACAGCGGCTA
1151 ACTTATCTGC CCCAGCCTTG GGGCAACTGC CGGGCGGAAA GCAAGCTCAG
30 1201 GGAGCCTGAG CTTCAGGGCT ACTCAGCCTA TAGTGTGTCT GCCTGCCGAC
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35 1301 CACATGCCAG GCAATGAGAC CATCTGCCCG CCAAATATCT ACATTGAATG
1351 TGCCGACCAC AACTGGACT CCCTGGGTGG GGGCTCTGAG GGCCCATGCT

5 1401 TCTGCCCTAC ACCCTGCAAC CTGACTCGTT ACGGCAAAGA GATCTCCATG
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1501 CCGCAATGAG ACCTACATAA GGGAAACTT CTTGGTCCTG GATGTCTTCT
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10 1601 TCAGCCTTGC TGGGGGACCT TGGGGGACAG ATGGGCCTGT TCATTGGGGC
1651 TAGCATCCTC ACCTTGCTGG AGATCCTTGA CTACATCTAT GAGGTCTCCT
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15 1751 TCCACTGGGG GCATCTCCAC TTTGGGGCTG CAGGAAGTGA AGGAACAGAG
1801 TCCCTGTCCA AATCGAGGCC GTGCTGAGGG TGGTGGGGCT AGCAACCTGC
1851 TTCCCAACCA TCACCACCCC CACGGCCCCC CAGGAAGCCT CTTTGAAAAC
20 1901 TTTGCTTGCT AGGATGGTGC TGTGTGGGGA AAGTACCCAT GAAACCCAC
1951 ACTCTCCTAT TCCTGGGACA GAAGGTCTGG GGCAGCCCAG GGCTAAGGGA
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2101 CCCAGGCAGG TGTCCAGGAA GGGCTAGAGA CCGGACTANG AGGCCCCCTGA
30 2151 GGAGGGGAGG GATGAAGAGA GGGAGGAAGG CGGAACCATG GTAGAGCCCC
2201 TCNGTACATT TGTATATATT TAGGGACTGG GTGGGGGTGG GACACAGACA
35 2251 TANAAGGTTT GGGCTGCAGG GGAGGGTGAC ACAGGATAGT CAGGGTCCCA
2301 ACCCTAATGG CANAANGCAA CTCCTTGGGA CCTAGGCATG TTGGGCTGGT

2351 TCTACTTCCC TCTTTCCAGG CCCAGCTCCC TCTTGGCATG GGGAGTGGGT
2401 GGCCCATCAG GCCTGGCCCA GCTCCCAN TT CCCCCTGTAC CAGCCCCACC
5 2451 ACAAGTTCCC TTCGTGGGGA GTGGGTGGAA ANACCTTTCA GACCTTGGCT
2501 AAGCTTATGG GGAGANGGAG TGGCCTTCTC ANGCTTGCT CCCTANAGAC
2551 TGGTTTTATA AANTGCTGGT GAACTTGGGA ATCNAGAGAC CCCNAGAAAA
10 2601 AAAAAAAAAA AAAAAAAAAA AA

15 (2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 539 amino acids
(B) TYPE: amino acid
20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SPASIC protein

25 1 MPPIEIVCKIK FAEDAKPKE KEAGDEQSL L GAAQGPAAPR DLATFASTST
51 LHGLGRACGP GPHGLRRTLW VLALLTSLAA FLYQAASLAR GYLTRPHLVA
101 MDPAAPAPVA GFPAVTL CNI NRFRHSALSD ADIFHLANLT GLPPKDRDGH
30 151 RAAGLRYPEP DMVDILNRTG HQLADMLKSC NFSGHHCSAS NFSVVYTRYG
201 KCYTFNADPQ SSLPSRAGGM GSGLEIMLDI QQEYLP IWR ETNETSFEAG
35 251 IRVQIHSQEE PPYIHQLGFG VSPGFQTFVS CQEQLTYLP QPWGN CRAES
301 KLREPELQGY SAYSVSACRL RCEKEAVLQR CHCRMVHMPG NETICPPNIY

5

351 IECADHTLDS LGGGSEGPCF CPTPCNLTRY GKEISMVKIP NRGSSARYLAR

401 KYNRNETYIR ENFLVLDVFF EALTSEAMEQ RAAYGLSALL GDLGGQMGLF

5 451 IGASILTLE ILDYIYEVSW DRLKRVWRRP KTPLRTSTGG ISTLGLQELK

501 EQSPCPNRGR AEGGGASNLL PNHHHPHGPP GSLFENFAC

10 (2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 526 amino acids

(B) TYPE: Protein

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: ASIC protein

20

SEQUENCE DESCRIPTION: SEQ ID NO: 3:

1 MELKTEEEV GGVQVSIQA FASSSTLHGL AHFSYERLS LKRALWALCF

51 LGSLAVLLCV CTERVQYYFC YHHVTKLDEV AASQLTFPAV TLCNLNEFRF

25

101 SQVSKNDLYH AGELLALLNN RYEIPDTQMA DEKQLEILQD KANFRSFKPK

151 PFNMREFYDR AGHDIRDMLL SCHFRGEACS AEDFKVVFTR YGKCYTFNSG

30

201 QDGRPRLKTM KGGTNGLEI MLDIQQDEYL PVWGETDETS FEAGIKVQIH

251 SQDEPPFIDQ LGFGVAPGFQ TfvSCQEQRL IYLPSPWGTC NAVTMSDFF

301 DSYSITACRI DCETRYLVEN CNCRMVHMPG DAPYCTPEQY KECADPALDF

35

351 LVEKDQEYCV CEMPCNLTRY GKELSMVKIP SKASAKYLAK KFNKSEQYIG

6

401 ENILVLDIFF EVLNYESIEQ KKAYEIAGLL GDIGGQMGLF IGASILTVLE

451 LFDYAYEVIK HRLCRRGKCQ KEAKRSSADK GVALSLDDVK RHNPCESLRG

5 501 HPAGMTYAAN ILPHHPARGT FEDFTC

10

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

15

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

20

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Rattus norvegicus

(F) TISSUE TYPE: Neurons

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

TAGCAGTGAGGCTCTGGCCAGTCCCA

25

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

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(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

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(vi) ORIGINAL SOURCE:

(A) ORGANISM: Rattus norvegicus

(F) TISSUE TYPE: Dorsal root ganglion

7

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CCAGACCTTCTGTCCCAGGAATAGG

5 (2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

15 (vi) ORIGINAL SOURCE:

(A) ORGANISM: Rattus norvegicus

(F) TISSUE TYPE: Dorsal root ganglion

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

20 GACCTGGCTACCTTTGCCAGCACCA

(2) INFORMATION FOR SEQ ID NO: 7:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

30 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Rattus norvegicus

35 (F) TISSUE TYPE: neurons

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

ATATGGGTAGGTATTCCTCCTGCTG

2) INFORMATION FOR SEQ ID NO: 8:

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(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

10

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Rattus norvegicus

15

(F) TISSUE TYPE: neurons

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

C/AGI,TAT/C,GG/CI,AAA/G,GAA/G,T/C/ATI,TCI,ATG

20

2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

25

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

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(vi) ORIGINAL SOURCE:

(A) ORGANISM: Rattus norvegicus

(F) TISSUE TYPE: neurons

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

35

IC/TT/A,IGC,ICC,IAT,A/GAA,IAG/A,ICC

JA

PCT09

RAW SEQUENCE LISTING

PATENT APPLICATION: US/09/701,747

DATE: 09/05/2001

TIME: 10:38:41

Input Set : A:\620-123.app

Output Set: N:\CRF3\09052001\I701747.raw

4 <110> APPLICANT: Wood, John N
5 England, Steven
6 Chen, Chih C
7 Akopian, Armen N
9 <120> TITLE OF INVENTION: Ion channels
11 <130> FILE REFERENCE: 620-123
13 <140> CURRENT APPLICATION NUMBER: US 09/701,747
C--> 14 <141> CURRENT FILING DATE: 2001-01-29
16 <150> PRIOR APPLICATION NUMBER: PCT/GB99/01743
17 <151> PRIOR FILING DATE: 1999-06-03
19 <150> PRIOR APPLICATION NUMBER: GB 9811965.4
20 <151> PRIOR FILING DATE: 1998-06-03
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p.5

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91 <213> ORGANISM: Rattus norvegicus
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97 Lys Pro Lys Glu Lys Glu Ala Gly Asp Glu Gln Ser Leu Leu Gly Ala
98 20 25 30
100 Ala Gln Gly Pro Ala Ala Pro Arg Asp Leu Ala Thr Phe Ala Ser Thr
101 35 40 45
103 Ser Thr Leu His Gly Leu Gly Arg Ala Cys Gly Pro Gly Pro His Gly
104 50 55 60
106 Leu Arg Arg Thr Leu Trp Val Leu Ala Leu Leu Thr Ser Leu Ala Ala
107 65 70 75 80
109 Phe Leu Tyr Gln Ala Ala Ser Leu Ala Arg Gly Tyr Leu Thr Arg Pro
110 85 90 95
112 His Leu Val Ala Met Asp Pro Ala Ala Pro Ala Pro Val Ala Gly Phe
113 100 105 110
115 Pro Ala Val Thr Leu Cys Asn Ile Asn Arg Phe Arg His Ser Ala Leu
116 115 120 125
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119 130 135 140
121 Lys Asp Arg Asp Gly His Arg Ala Ala Gly Leu Arg Tyr Pro Glu Pro
122 145 150 155 160

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RAW SEQUENCE LISTING

DATE: 09/05/2001

PATENT APPLICATION: US/09/701,747

TIME: 10:38:42

Input Set : A:\620-123.app

Output Set: N:\CRF3\09052001\I701747.raw

I701747-09052001

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127 Leu Lys Ser Cys Asn Phe Ser Gly His His Cys Ser Ala Ser Asn Phe
128                               180                185                190
130 Ser Val Val Tyr Thr Arg Tyr Gly Lys Cys Tyr Thr Phe Asn Ala Asp
131                               195                200                205
133 Pro Gln Ser Ser Leu Pro Ser Arg Ala Gly Gly Met Gly Ser Gly Leu
134                               210                215                220
136 Glu Ile Met Leu Asp Ile Gln Gln Glu Glu Tyr Leu Pro Ile Trp Arg
137 225                               230                235                240
139 Glu Thr Asn Glu Thr Ser Phe Glu Ala Gly Ile Arg Val Gln Ile His
140                               245                250                255
142 Ser Gln Glu Glu Pro Pro Tyr Ile His Gln Leu Gly Phe Gly Val Ser
143                               260                265                270
145 Pro Gly Phe Gln Thr Phe Val Ser Cys Gln Glu Gln Arg Leu Thr Tyr
146                               275                280                285
148 Leu Pro Gln Pro Trp Gly Asn Cys Arg Ala Glu Ser Lys Leu Arg Glu
149                               290                295                300
151 Pro Glu Leu Gln Gly Tyr Ser Ala Tyr Ser Val Ser Ala Cys Arg Leu
152 305                               310                315                320
154 Arg Cys Glu Lys Glu Ala Val Leu Gln Arg Cys His Cys Arg Met Val
155                               325                330                335
157 His Met Pro Gly Asn Glu Thr Ile Cys Pro Pro Asn Ile Tyr Ile Glu
158                               340                345                350
160 Cys Ala Asp His Thr Leu Asp Ser Leu Gly Gly Gly Ser Glu Gly Pro
161                               355                360                365
163 Cys Phe Cys Pro Thr Pro Cys Asn Leu Thr Arg Tyr Gly Lys Glu Ile
164                               370                375                380
166 Ser Met Val Lys Ile Pro Asn Arg Gly Ser Ala Arg Tyr Leu Ala Arg
167 385                               390                395                400
169 Lys Tyr Asn Arg Asn Glu Thr Tyr Ile Arg Glu Asn Phe Leu Val Leu
170                               405                410                415
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173                               420                425                430
175 Ala Tyr Gly Leu Ser Ala Leu Leu Gly Asp Leu Gly Gly Gln Met Gly
176                               435                440                445
178 Leu Phe Ile Gly Ala Ser Ile Leu Thr Leu Leu Glu Ile Leu Asp Tyr
179                               450                455                460
181 Ile Tyr Glu Val Ser Trp Asp Arg Leu Lys Arg Val Trp Arg Arg Pro
182 465                               470                475                480
184 Lys Thr Pro Leu Arg Thr Ser Thr Gly Gly Ile Ser Thr Leu Gly Leu
185                               485                490                495
187 Gln Glu Leu Lys Glu Gln Ser Pro Cys Pro Asn Arg Gly Arg Ala Glu
188                               500                505                510
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191                               515                520                525
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RAW SEQUENCE LISTING

DATE: 09/05/2001

PATENT APPLICATION: US/09/701,747

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199 <211> LENGTH: 526

200 <212> TYPE: PRT

201 <213> ORGANISM: Rattus norvegicus

203 <400> SEQUENCE: 3

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208           20           25           30
210 Ile Phe Ser Tyr Glu Arg Leu Ser Leu Lys Arg Ala Leu Trp Ala Leu
211           35           40           45
213 Cys Phe Leu Gly Ser Leu Ala Val Leu Leu Cys Val Cys Thr Glu Arg
214           50           55           60
216 Val Gln Tyr Tyr Phe Cys Tyr His His Val Thr Lys Leu Asp Glu Val
217   65           70           75           80
219 Ala Ala Ser Gln Leu Thr Phe Pro Ala Val Thr Leu Cys Asn Leu Asn
220           85           90           95
222 Glu Phe Arg Phe Ser Gln Val Ser Lys Asn Asp Leu Tyr His Ala Gly
223           100          105          110
225 Glu Leu Leu Ala Leu Leu Asn Asn Arg Tyr Glu Ile Pro Asp Thr Gln
226           115          120          125
228 Met Ala Asp Glu Lys Gln Leu Glu Ile Leu Gln Asp Lys Ala Asn Phe
229           130          135          140
231 Arg Ser Phe Lys Pro Lys Pro Phe Asn Met Arg Glu Phe Tyr Asp Arg
232 145           150          155          160
234 Ala Gly His Asp Ile Arg Asp Met Leu Leu Ser Cys His Phe Arg Gly
235           165          170          175
237 Glu Ala Cys Ser Ala Glu Asp Phe Lys Val Val Phe Thr Arg Tyr Gly
238           180          185          190
240 Lys Cys Tyr Thr Phe Asn Ser Gly Gln Asp Gly Arg Pro Arg Leu Lys
241           195          200          205
243 Thr Met Lys Gly Gly Thr Gly Asn Gly Leu Glu Ile Met Leu Asp Ile
244           210          215          220
246 Gln Gln Asp Glu Tyr Leu Pro Val Trp Gly Glu Thr Asp Glu Thr Ser
247 225           230          235          240
249 Phe Glu Ala Gly Ile Lys Val Gln Ile His Ser Gln Asp Glu Pro Pro
250           245          250          255
252 Phe Ile Asp Gln Leu Gly Phe Gly Val Ala Pro Gly Phe Gln Thr Phe
253           260          265          270
255 Val Ser Cys Gln Glu Gln Arg Leu Ile Tyr Leu Pro Ser Pro Trp Gly
256           275          280          285
258 Thr Cys Asn Ala Val Thr Met Asp Ser Asp Phe Phe Asp Ser Tyr Ser
259           290          295          300
261 Ile Thr Ala Cys Arg Ile Asp Cys Glu Thr Arg Tyr Leu Val Glu Asn
262 305           310          315          320
264 Cys Asn Cys Arg Met Val His Met Pro Gly Asp Ala Pro Tyr Cys Thr
265           325          330          335
267 Pro Glu Gln Tyr Lys Glu Cys Ala Asp Pro Ala Leu Asp Phe Leu Val
268           340          345          350
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RAW SEQUENCE LISTING

DATE: 09/05/2001

PATENT APPLICATION: US/09/701,747

TIME: 10:38:42

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Output Set: N:\CRF3\09052001\I701747.raw

T06210.447060

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276 Ala Lys Tyr Leu Ala Lys Lys Phe Asn Lys Ser Glu Gln Tyr Ile Gly
277 385          390          395          400
279 Glu Asn Ile Leu Val Leu Asp Ile Phe Phe Glu Val Leu Asn Tyr Glu
280          405          410          415
282 Thr Ile Glu Gln Lys Lys Ala Tyr Glu Ile Ala Gly Leu Leu Gly Asp
283          420          425          430
285 Ile Gly Gly Gln Met Gly Leu Phe Ile Gly Ala Ser Ile Leu Thr Val
286          435          440          445
288 Leu Glu Leu Phe Asp Tyr Ala Tyr Glu Val Ile Lys His Arg Leu Cys
289          450          455          460
291 Arg Arg Gly Lys Cys Gln Lys Glu Ala Lys Arg Ser Ser Ala Asp Lys
292 465          470          475          480
294 Gly Val Ala Leu Ser Leu Asp Asp Val Lys Arg His Asn Pro Cys Glu
295          485          490          495
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342 <211> LENGTH: 24
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344 <213> ORGANISM: Rattus norvegicus

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Use of n and / or Xaa has been detected in the Sequence Listing. Review the Sequence Listing to ensure a corresponding explanation is present in the <220> to <223> fields of each sequence using n or Xaa.

VERIFICATION SUMMARY

DATE: 09/05/2001

PATENT APPLICATION: US/09/701,747

TIME: 10:38:43

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L:78 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:1

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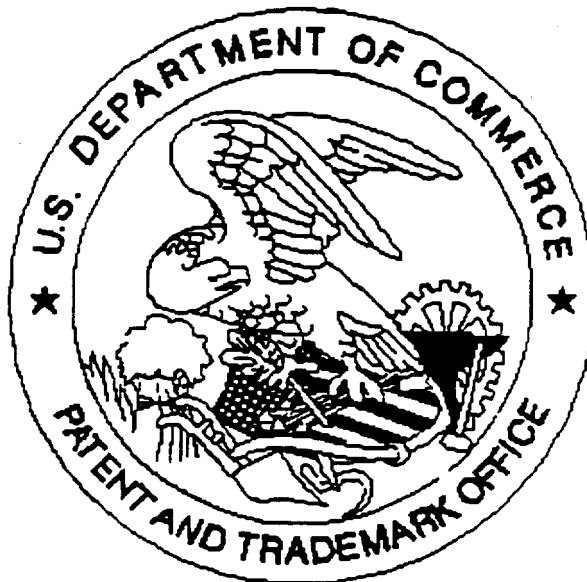
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I701747.09052001

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SCANNED # 24

Application deficiencies found during scanning:

☒ Page(s) 7 of 7 of Sequence Listing were not present
for scanning. (Document title)

☐ Page(s) _____ of _____ were not present
for scanning. (Document title)

☐ *Scanned copy is best available.*